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LABORATORY AND FIELD EVALUATION OF A WATERLESS FOOD SERVICE SANITATION SYSTEM USED BY MILITARY MOBILE KITCHEN TRAILER CREWS

By
Edmund M. Powers
Andrea Hoikala
Michael Cioffi
Claire Lee

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UNITED STATES ARMY NATICK
RESEARCH, DEVELOPMENT AND ENGINEERING CENTER
NATICK , MASSACHUSETTS 01760-5000

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Edmund M. Powers, Andrea Hoikala,
Michael Cioffi and Claire Lee

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13. ABSTRACT *(Maximum 200 words)*

The feasibility and efficacy of a waterless sanitation system (WSS) for cleaning food service utensils and removing bacteria were demonstrated in the laboratory and tested in the field under actual conditions during Army training exercises with Mobile Kitchen Trailers (MKT). The WSS employed three wipes used in sequence: 1) a detergent/degreaser wipe; 2) a deionized water wipe to rinse the surface; 3) a Quat disinfectant/sanitizer wipe. *Escherichia coli* and *Staphylococcus aureus* cells in biofilms produced on food-soiled, stainless steel surfaces were reduced by 99.999% to 100% at 5° C and 26° C by the WSS. The sanitizer dilution used reduced planktonic cells of severalfood poisoning bacteria by more than six logs. In the field, all surfaces of food-serving utensils and equipment examined were monitored for bacterial contamination by using Difco Hycheck™ contact slides. Coliform bacteria were assessed by using Millipore™ swab test kits. Total bacteria, coliforms and molds on all utensils and equipment cleaned and sanitized by the WSS were reduced far below the U.S. Public Health Service limit for food contact surfaces. Utensils, equipment and greasy fry grills were cleaned very easily and effectively by the WSS. A waterless food service sanitation capability will give the MKT an emergency backup cleaning and sanitation system when either hot water or a potable water supply is unavailable.

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PREFACE

A "waterless" sanitation system (WSS) was developed to support Mobile Kitchen Trailers (MKT) in the field (1). Nondevelopmental items were used. Laboratory studies demonstrated that when water is not available to clean and sanitize food service equipment and utensils a set of specially prepared towelettes or wipes can be used as an emergency substitute.

This project was a Military Service Requirement, MSR AM93-7, supported by both the U.S. Army and U.S. Marine Corps under project #1E463747D610, Food Advanced Development, Task #D610XX. This study started in October 1992 and was completed in October 1993.

The wipes are undergoing confirmatory testing required by the U.S. Environmental Protection Agency (EPA), at the Texwipe Co., Upper Saddle River, NJ, in order to qualify for and obtain EPA registration. A Purchase Order was awarded to the Texwipe Co. on September 28, 1993 for delivery in December, 1993. The tests include verification of sterility, storage stability of active ingredients, and package integrity and compatibility. The wipes will be packaged and sterilized by irradiation, and once registered will be available for purchase. A Commercial Item description is expected to be completed by May 1994. The wipes will be fielded as a Common Table of Allowance (CTA) 50-970 item.

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LABORATORY AND FIELD EVALUATION OF A WATERLESS SANITATION SYSTEM USED BY MILITARY MOBILE KITCHEN TRAILERS

INTRODUCTION

The biocidal efficacy and feasibility of a waterless (towellette) sanitation system (WSS) for cleaning and sanitizing stainless steel surfaces was documented in 1992 (1). Tests demonstrated that Escherichia coli and Staphylococcus aureus in biofilms produced on food-soiled stainless steel surfaces were reduced by the WSS by 99.999% to 100%. The WSS employed three wipes used in sequence. The first wipe contained a detergent/degreaser called Vestapower (Calgon Corporation, Pittsburg, PA). The second wipe contained deionized water to rinse the surface. The third wipe contained a quaternary ammonium sanitizer called Product QDS (Calgon Vestal Laboratory, St Louis, MO) formerly called Syn-Cide Plus (1).

The WSS was developed to support military Mobile Kitchen Trailers (MKT) in the field. Because water may not be readily available in all theaters and scenarios, a waterless (towelette) food service sanitation capability will give the MKT an emergency backup sanitation system when either hot water or a potable water supply is unavailable or must be conserved for cooking and drinking.

This report continued the studies initiated in fiscal year 1992 and completes the evaluation of the WSS developed at Natick by combining and validating commercially available items (1). The bactericidal efficacy of Product QDS against several foodborne pathogenic bacteria was compared to a fatty acid sanitizer called Mandate (Klenzade, St. Paul, MN). In addition, the prototype wipes for the WSS were custom produced, packaged and sterilized by the Texwipe Co., Upper Saddle River, NJ, and evaluated by soldiers in the field at Fort Lee, VA.

MATERIALS AND METHODS

Materials and methods used were the same as previously reported (1) with the following additions:

Determining efficacy of sanitizers

a. Planktonic cells

Reagents, preparation of stock culture and operating technique were according to Association of Official Analytical Chemists (AOAC) Official Methods of Analysis, section 960.09, 1990 (2). All cultures were activated by three daily tranfers on nutrient agar (Difco Laboratory, Detroit, MI). One mL of a standardized suspension of planktonic cells (1×10^{10}/mL) was exposed to Product QDS (Syncide plus) and Mandate sanitizers for 30 seconds (2,3). At the end of the time period the cells were immediately transferred to neutralizing buffers to inactivate the sanitizers. Dilutions were also made in neutralizing buffers and plate counts were made in neutralizing agar pour plates (Difco).

b. Biofilm bacteria

Bacteria exposed to sanitizers on stainless steel chips were suspended in 10% skim milk (Difco). The chips were inoculated with 10^6 cells by evenly spreading 0.01 mL of the milk suspension on them. The chips were dried at room temperature for one hour before immersing them in the recommended dilution of the sanitizer. Biofilm cells on stainless steel chips were exposed to the sanitizers for ten minutes (2,3). Bacteria were recovered by swabbing the surface (4). The swab was deposited in neutralizing buffer to inactivate the sanitizer and dilutions were also made in neutralizing buffer. Plate counts were made on neutralizing agar pour plates (Difco). Surfaces of stainless steel frying pans soiled by food inoculated with test bacteria were sampled for bacterial counts, cleaned and sanitized as previously reported (1).

Inactivation of sanitizers

To avoid bacteriostic conditions in growth media, the QAC in Product QDS in which cells were suspended was inactivated by transferring 1 mL of the treated cell suspension to 9 mL of neutralizing buffer (Difco) or to Millipore (Bedford, MA) buffer sets (18 mL)(5). Mandate, a fatty acid sanitizer, was inactivated in Sorensons buffer (6) or Millipore buffer sets. Swabs were likewise inactivated. In the field residual sanitizers on surfaces sampled were inactivated by the D/E neutralizing agar in the Hycheck contact slides (Difco) which allowed the bacteria recovered to grow.

Test bacteria

Test bacteria included Bacillus cereus, B6Ac; Escherichia coli, ATCC 11229 (2); Klebsiella terrigena, ATCC 33257; Listeria monocytogenes, N2-1; Pseudomonas aeruginosa, QM-3-1517; Staphylococcus aureus, ATCC 6538 (2); Streptococcus faecalis, ATCC 19433; and Salmonella typhimurium, ATCC 14028.

Assessing the microbiological contamination on food contact surfaces in the field.

All surfaces of food-serving utensils and equipment examined in the field were monitored for bacterial contamination by using Hycheck contact slides containing D/E neutralizing agar on both sides (Difco). Coliform bacteria were assessed by using Millipore swab test kits (5).

Stainless steel chips

Stainless steel chips (2"L by 7/8"W) were fabricated from #304 steel. The chips were autoclaved in alconox detergent, sonicated, brushed, rinsed in tap water, soaked in acetone, soaked in boiling distilled water, rinsed 3 times in tap water, rinsed 3 times in deionized water, soaked in absolute ethyl alcohol, and air dried. The chips were sterilized by autoclaving for 30 minutes at 121°C.

Determination of concentration of quarternary ammonium compound (QAC)

a. Bromophenol blue method

The concentration of QAC in Product QDS (Syncide Plus) (1) was determined by a bromophenol blue method (7). Add 25 mL of chloroform, 25 mL salt buffer solution (7 g sodium carbonate, 100 g sodium sulfate and 1000 mL distilled water, pH10) and three drops of 0.1% bromophenol blue indicator to 50 mL of sample in a 250 mL flask. Stopper the flask and shake vigorously. The mixture was titrated with 0.003 N sodium lauryl sulfate dropwise. The endpoint was the first definite appearance of a violet color in the upper layer when viewed under direct light. The ppm QAC was calculated by the following formula:

$$\frac{(\text{mL of } NaLSO_4)(\text{N of } NaLSO_4)(MW)\ (1000)}{(\text{mL of sample})}$$

b. QT-30 test paper

The test kit is available through customer service, Calgon Vestal Laboratories, St Louis, MO 63166.

RESULTS

Table 1 shows the reduction of E. coli and S. aureus by the WSS in biofilms produced on food-soiled stainless steel frying pan surfaces. The bacteria grew to billions per gram and spoiled the food. The surfaces and wipes were equilibrated at 5 °C and 26 °C before application. Reduction of bacteria ranged from 99.98% to 100% at 5 °C and from 99.999% to 100% at 26 °C. The percent reduction was compared to counts obtained before application of the WSS (1).

Table 1. Efficiency of the waterless sanitation system (towellettes) in the removal of bacteria in biofilms produced on stainless steel surfaces by E. coli and S. aureus in selected foods[a].

	Average percent bacterial reduction[b] by WSS[c]	
RATION	5°C	26°C
Pork chow mein	99.9993	100.00
Beef stew	99.98	100.0
Chicken ala king	99.999	99.9994
Chicken stew	100.0	99.999
Corn beef hash	99.99	100.0
Escal. potatoes	100.0	99.9993
Tuna and noodles	99.9998	100.0
Skim milk	——	99.99996

[a]Cultures of E. coli (EC) and S. aureus (SA) were inoculated as pure cultures and/or mixed in equal volumes. One million cells were added to 100 g of food (10,000/g) that was spread over the entire surface of 12"by 12" stainless steel pans. The soiled pans were then incubated at 35°C for 24 hours to encourage bacterial growth and food spoilage. Counts in the spoiled food exceeded 10^9/g.

[b]Reduction was compared to counts obtained before application of the WSS.

[c]WSS - Wipe #1 contained Vesta Power detergent; wipe #2 contained deionized water; wipe #3 contained Product QDS (formerly Syn-Cide Plus) sanitizer.

Selection of the proper towel material for the wipes is very important to avoid inactivating the QAC. Table 2 shows that cellulose (paper and cotton) towels reduced the QAC in the Product QDS sanitizer by 53% to 95%. Polypropylene and polyester reduced the QAC by only 8% to 30%, respectively. Therefore, polypropylene or polyester material must be used for sanitizer wipes containing QAC's. Since some inactivation of the QAC can be expected the sanitizer must be formulated overstrength to achieve the desired concentration of QAC in the wipe. The Product QDS wipe must contain 150 ppm QAC.

Table 2. Inactivation of quaternary ammonium compound (QAC) in Product QDS by towel material.

Towel	Composition	Average[a] Percent reduction of QAC
Kim towel	Cellulose	53
Sturdi-wipe	Cellulose	89
Webril towel	Cellulose	95
Texwipe 60/40	Polyester/cellulose	51
Army cloth	Polypropylene	8
Army cloth	Polyester	18
Texwipe	Polyester	20
Exsorbx 400	Polyester	30

[a] Average of two to seven trials.

Table 3 compares the efficacy of QDS and Mandate sanitizers on planktonic cells (cells in suspension) of 7 foodborne pathogens and K. terrigena, an environmental coliform organism. A five-log reduction (99.999%) of bacteria within 30 seconds was considered effective (2,3). The QDS was more effective than Mandate and achieved more than a six-log reduction within 30 seconds, of all bacteria except the sporeforming B. cereus. Failure to destroy sporeformers was not unexpected.

Table 3. Bactericidal efficacy of Product QDS and Mandate sanitizers on planktonic cells[a].

	Average log reduction after 30 seconds	
Bacteria[b]	QDS[c]	Mandate[d]
B. cereus (sporeformer)	1.3	1.3
E. coli	>6	>6
K. terrigena	>6	>5.3
L. monocytogenes	>6	>6
P. aeruginosa	>6	>6
S. aureus	>6	>6
S. faecalis	>6	>6
S. typhimurium	>6	>4.6

[a] Cells in suspension, AOAC procedure, 15th ed., page 138, 1990 (4).

[b] Approximately 10 x 10^9 cells/mL exposed to sanitizers (4).

[c] Product QDS is a quarternary ammonium disinfectant sanitizer.

[d] Mandate is a fatty acid sanitizer.

Product QDS also reduced biofilm cells of S. aureus on 1"by 2" stainless steel chips by 99.999%, after 10 minutes, compared to only 99.3% reduction by the Mandate sanitizer (2,3). E. coli, however, did not survive the 1 hour drying time at room temperature on stainless steel chips under the same conditions (2,3) as shown in Figure 1. Only 24% of the cells were recovered after 30 minutes and 4% after one hour.

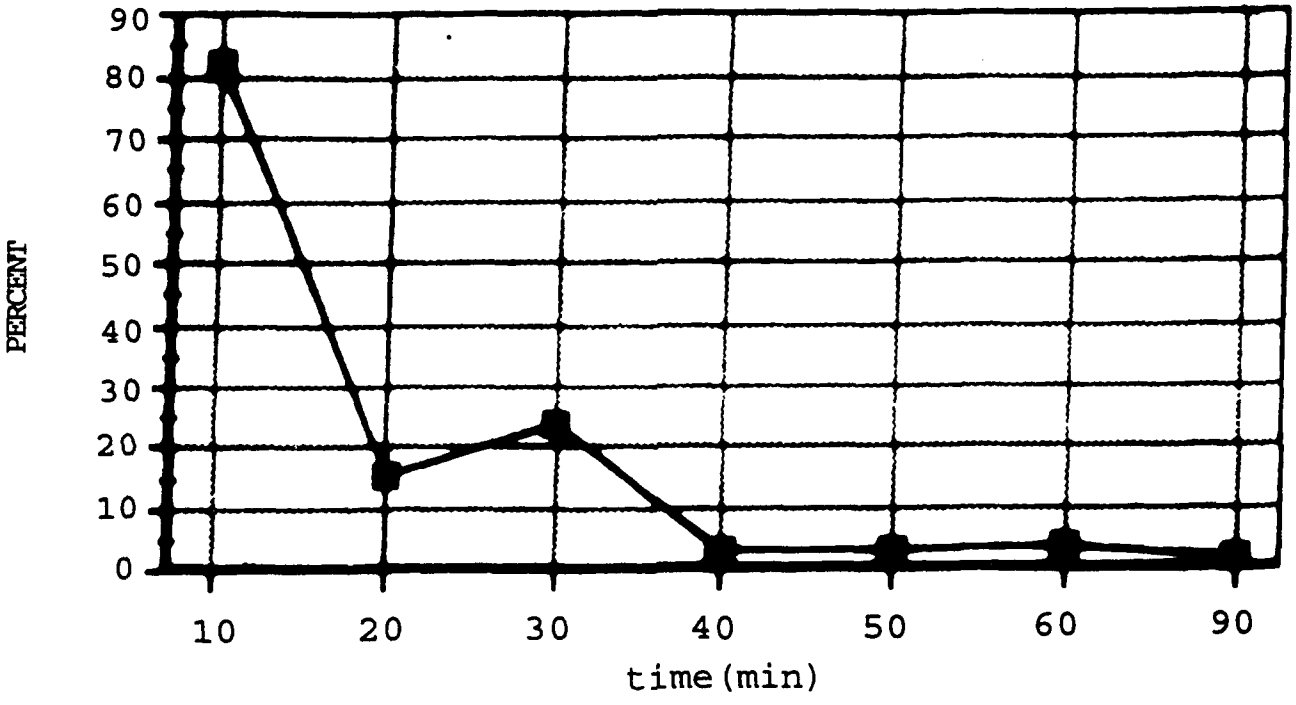

Figure 1. Rate of decrease of E. coli in a skim milk biofilm on stainless steel at 26°C.

The results obtained during the field evaluation at Fort Lee are shown in Tables 4 to 8. The meals attended were two breakfasts and two dinners. The foods served at each meal are shown in Table 4.

TABLE 4. Foods served at two breakfast and dinner meals

Meal	Breakfast	Dinner
1	Scrambled eggs	Beef stew
	Grits	Rice
	Potatoes	Green beans
	Spam	Cake
2	Creamed ground beef (TP)	Pork
	Egg & sausage omelet (TP)	Mashed potatoes
	Grits	Gravy
	Cake	Corn
		Peach cobbler

The utensils cleaned and sanitized by the WSS after each meal are shown in Table 5.

TABLE 5. Serving utensils cleaned and sanitized with the waterless sanitation system (wipes) after breakfast and dinner meals.

Utensil	Breakfast	Dinner
Cake cutter	1	2
Fork	0	1
Ice cream scoop	0	1
Knife	1	1-2
Ladle (quart)	1-2	0
Ladle (small)	1	1
Skimmer	1	1
Spatulas	1-2	1-2
Spoons	4	1
Thongs	0	1-3
Whisk	1	0

To establish a baseline for sanitation of the utensils in the field, the bioburden of utensils cleaned and sanitized by standard Army field sanitation procedures (8) was determined before and after application of the WSS. Table 6 shows that the WSS system reduced the total count on the utensils, previously sanitized by standard Army procedures, from 9.2/in^2 to only 2/in^2 on average. This represents a reduction of total colony forming units (CFU's) from 184 to 42, shown in parenthesis. Molds were reduced from 4 to 0.06/in^2. Coliform bacteria were not detected before or after applying the WSS. Utensils cleaned by standard Army procedures before application of the WSS were acceptable and in compliance with U.S. Public Health Service (PHS) requirements of total counts not greater than 12.5/in^2(4).

TABLE 6. Bioburden of ten utensils sanitized by the standard military field sanitation procedure before and after application of the waterless sanitation system (wipes).

WSS	No. of samples	Average CFU/in^2		
		Total[a]	Molds	Coliforms
Before	20	9.2 (184)[b]	4 (75)	0
After	20	2.0 (42)	0.06 (1).	0

[a]Total count includes bacteria and mold colony forming units (CFU)/in^2. Acceptable total CFU for sanitized surfaces is 12.5/in^2 (3).

[b]( ) = Total CFU on 20 surface areas sampled.

Table 7 shows the bacterial and mold counts on foodservice utensils used during breakfast and dinner meals after applying the WSS. All counts were far below the 12.5 CFU's/in^2 considered acceptable on sanitized surfaces (4). Molds were reduced to less than one/in^2 and coliforms were absent.

TABLE 7. Bacterial and mold counts on foodserving utensils after cleaning and sanitizing soiled utensils by the WSS.

No. utensils	No. samples	Meal	Average CFU/in^2		
			Total[a]	Molds	Coliforms
10	20	B	4.0	0.2	0
11	22	B	0.73	0	0
11	22	D	3.6	0.2	0
10	20	D	2.6	0.5	0

[a]Total count includes bacteria and mold colony forming units (CFU)/in^2. Acceptable total CFU for sanitized surfaces is 12.5/in^2 (3).

The WSS was also very effective in sanitizing foodservice equipment as shown in Table 8. Microbiological samples were taken from surfaces before and after wiping with the WSS. Counts on all equipment surfaces were reduced below the maximum of 12.5 CFU's/in^2 allowed, with one exception. A table top sampled after the first dinner may have been inadequately cleaned and sanitized. The tent was poorly lighted, and it was too dark to see the surface clearly. More importantly, it is suspected that the soldier did not apply the sanitizing wipe for the required 30 seconds. However, the reduction of the total colony count after applying the WSS was still substantial, going from too numerous to count (TNTC) to only 48 CFU's/in^2. The WSS was also very effective on the large cake pan and frying grill. The total count/in^2 on the grill was reduced from 20 to less than 1. The grease was completely removed and undiscernible to touch and sight.

TABLE 8. Bioburden on soiled foodservice equipment and selected utensils before and after application of the waterless sanitation system (wipes).

		Average CFU/in^2			
		Before WSS		After WSS	
Equipment	Meal[a]	Total	Molds	Total	Molds
Cake pan	D	47	0	8	1
Countertop (MKT)	B	10	0	8	2
Grill (greasy)	B	20	1	0.3	0
Pot (mashed potatoes)	D	—	—	3	0.5
Pot (rice)	D	—	—	6	0
Serving spoon (grits)	B	11	0	2	0.3
Table Top	B	TNTC[b]	0.25	2	0
Table Top	D	TNTC	4	48	1

[a]D = Dinner; B = Breakfast
[b]Too numerous to count (>200 CFU's)

DISCUSSION

As demonstrated, the WSS was very effective in meeting and often exceeding the required reduction of test bacteria (2,3) in biofilms produced on stainless steel surfaces (see also Powers, 1992). The QDS sanitizer tested alone effectively killed planktonic cells of seven foodborne bacterial pathogens and K. terrigena within 30 seconds.

The WSS was also very effective under field conditions when used by soldiers to clean and sanitize surfaces of soiled foodservice utensils and equipment. The total CFU's on all WSS sanitized utensils and equipment were below the PHS standard (4) of less than 12.5 CFU's/in^2. The detergent wipe was very effective in removing grease from the grill as well as removing a mixture of fuel and grease on the stainless steel surface under the grill in the MKT. Dried and burnt foods on surfaces were also effectively removed by the detergent wipe. Removal of such dried foods from surfaces can be expedited by first wetting the surface with the detergent wipe and then applying a dry scouring pad as one would do if water was used. A dry towel was also used to remove gross food residues from all surfaces before using the WSS, in order to save the wipes. In many cases one wipe was used for several utensils depending on the utensils size and condition. Only one wipe of each type was required to clean and sanitize a table top (4'by 3'). When the detergent wipe produced an excess of suds due to prolonged wiping, excessive detergent, or because it was too large for the surface, a dry towel was used to remove the suds before the rinse wipe was applied. However, if necessary, more than one rinse wipe can be used. Such modifications in use of the WSS will be included in the MANPRINT instructions which will be supplied with the WSS.

The sanitizer wipe, in addition to being biocidal, polished the utensils and surfaces in the MKT. Surfaces sanitized by the WSS "shined" more than those sanitized by standard procedures. This was most likely due to the fact that the detergent water used in the standard Army field-washing procedure (8) was too hot for hand washing. Hot water causes the proteins from food residues to "bake" on the utensil surface, producing a film that dulls the surface and is difficult to remove. Therefore, detergent water temperature for manual washing should be only as hot as the hands can stand, which ranges from 110 °F to 125 °F (9,10). Wash temperatures specified by the Army Field Manual 21-10-1 range from 120°F to 150°F (8). Thermometers should be provided to permit frequent checks of water temperature when water is used as the sanitizing agent (4). Another advantage of the WSS is that thermometers to check temperatures are not needed since the detergent and sanitizing wipes are effective even at low temperatures.

The WSS was developed to provide the MKT with an emergency back-up system to clean and sanitize utensils when water is not available. However, the wipes of the WSS may have a broader application in an emergency situation as was demonstrated during the field trial. The WSS sucessfully cleaned several equipment surfaces and countertops including a grill in the MKT as well as large pots and pans. Therefore, the WSS wipes could also be used in an abbreviated emergency mode to clean and sanitize essential food contact surfaces and equipment in the MKT until a water supply was restored, thus allowing the MKT to complete its mission. The wipes could also be used for many surfaces in a traditional foodservice facility, and certainly in a civilian application by campers when hot water and detergents are unavailable.

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